

## **A Reevaluation of the Phylogeography of the Chattahoochee Slimy Salamander (*Plethodon chattahoochee*) Using Next-Generation Genomic Data**

The *Plethodon glutinosus* species complex is a group of large-bodied, lungless salamanders that are widespread across the eastern United States. Species in this group are morphologically cryptic and were originally delimited using allozyme data, but in most cases, the extent of contemporary gene flow between named taxa has not been rigorously evaluated. The Appalachian Mountains of northern Georgia are home to several members of this group—including *P.*

*glutinosus*, *P. teyahalee*, *P. chlorobryonis*, and *P. chattahoochee*. *P. chattahoochee* has been found to be especially cryptic both morphologically and genetically as both intraspecific genetic variation and species boundaries within the *P. glutinosus* complex have been poorly defined in the past. Previous mitochondrial (mtDNA) data have suggested: 1) the potential paraphyly of *P. chattahoochee* with respect to *P. teyahalee*; 2) notable geographic variation within *P.*

*chattahoochee* (informally described as Northern, Southern, and Western clades). In order to further define genetic diversity and evaluate species boundaries in *P. chattahoochee*, we collected and assembled next-generation sequencing (3RAD) data from >40 individuals of this species—spanning its entire geographic distribution, all mtDNA clades, and a hybrid zone with the more distantly related *P. shermani*—and representatives of four other species in the *P.*

*glutinosus* species complex. We then conducted a variety of phylogenomic and population genomic analyses using the ipyrad genomic analysis toolkit on a high-performance computing cluster. Our results demonstrate the importance of multilocus genomic datasets for identifying gene flow among cryptic species, and we discuss their broader taxonomic implications within the *P. glutinosus* complex.

Keywords: phylogenomic analysis, population genomic analysis, cryptic speciation, next-generation genomic data, phylogeography, intraspecific genetic variation, species boundaries, multilocus genomic datasets, gene flow, phylogenetics